Table 4. Phytotoxic activity of the triazine **33** against atrazine-resistant and wild-type *Chenopodium album*

		Phytotoxic activity[b]	
Compound[a]	Rate (g AI ha^{-1})	Resistant Type[c]	Wild-type[d]
33	6.25	4	5
	12.50	5	5
	25.0	5	5
Atrazine	6.25	1	5
	12.50	1	5
	25.0	1	5
	800	1	5

[a] Applied as 100 g kg^{-1} WP.
[b] On a scale 0–5, where 0 = no effect, 5 = complete kill, assessed 12 days after treatment.
[c] 2.2–2.4 leaves, 4.5 cm.
[d] 2.5–2.7 leaves, 5.5 cm.

concentration (10^{-7} M), while a relatively high concentration (10^{-6} M) of compound **31** (2-Cl) was needed. A steric factor may cause its lower affinity (unpublished results).

PET inhibitory activities of the triazine compounds were evaluated by using thylakoids from wild-form and atrazine-resistant *C. album*. The pI_{50} values for compound **33** were 7.34 and 7.43 for wild **W** and resistant **R** types, respectively, indicating the anti-resistant nature of the compound ($I_{50}\mathbf{R}/I_{50}\mathbf{W} = 0.8$). Considering this anti-resistant nature, together with the result obtained from the binding experiment, triazine **33** may have binding partners (amino acids) different from atrazine at D1 protein, as was reported for diuron.[6]

Finally, post-emergence phytotoxic activity of compound **33** was evaluated against both atrazine-resistant mutant and wild-type *C. album* (Table 4). Both the atrazine-resistant mutant and the wild-type grown in a greenhouse were well controlled by the triazine **33**, although the mutant was not killed by atrazine, even at a dose far exceeding the conventional use rate (1–4 kg ha^{-1}). The triazine **33** may be a promising alternative to atrazine where resistence to the latter exists.

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Activity of the ilicicolins against plant pathogenic fungi

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Abstract: Ilicicolins D, E, F, dechloroilicicolin D, ascofuranone and arthrichitin were isolated from the fermentation broth of *Nectria* sp (HIL Y 90 3333). The ilicicolins showed good fungicidal activity *in planta*.

Keywords: Ilicicolins; microbial metabolites; fungicidal; *Nectria* sp

1 INTRODUCTION

Fungal attacks on crops reduce harvest yields each year, and some US$5 500 million was spent in 1997 on chemical control of fungal diseases. There is a continuing need for new fungicides to provide improved levels of control and solutions to new problems, and natural products can provide novel leads for these, as exemplified by the strobilurins that led to the β-methoxyacrylates. In the course of our screening for fungicidal agents from micro-organisms, the ilicicolins (Fig 1; **1**–**4**) were isolated from a fungicidally active fermentation broth of a fungal culture of *Nectria* sp, HIL Y 90 3333. Although these compounds had been isolated previously,[1,2] their effects against plant pathogenic fungi *in planta* have not been widely reported.[3] This report describes the fermentation, isolation and fungicidal activity of the ilicicolins, along with the other metabolites isolated.

The fungal strain Y 90 3333 was isolated from a soil

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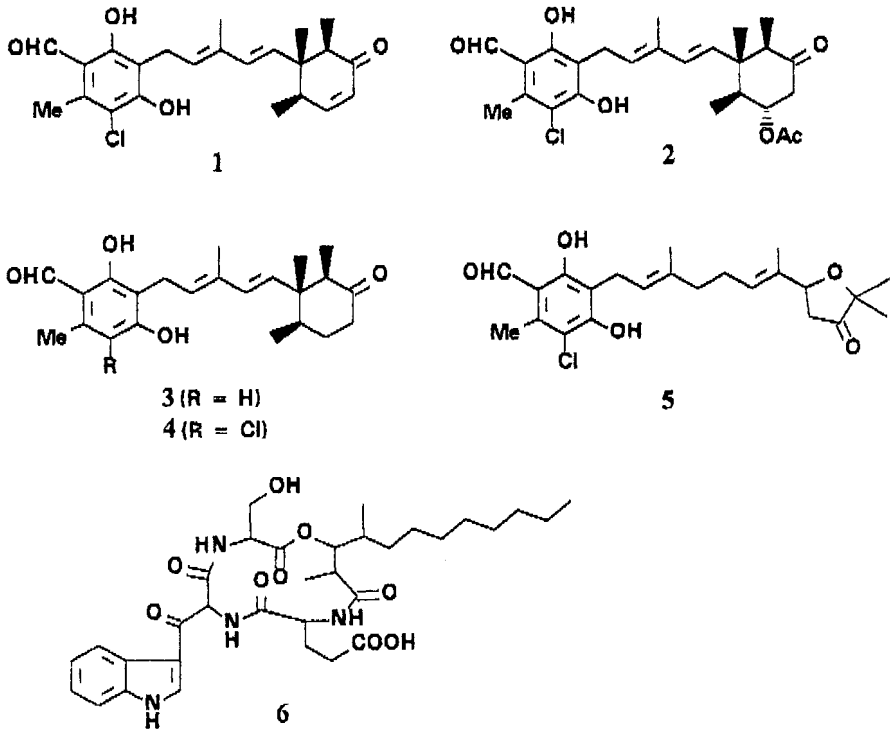

Figure 1. Compounds discussed in text.

sample collected near the Kabini River in India using a soil plate method and was identified as a *Nectria* sp at the German Collection of Micro-organisms and Cell Cultures, Braunschweig, Germany (DSM No 10658). This culture co-produced the ilicicolins (**1**–**4**), the related metabolite ascofuranone (**5**)[4] and arthrichitin (**6**),[5] all contributing to the fungicidal activity of the broth.

2 EXPERIMENTAL

2.1 Extraction

Flasks containing sterile seed medium [100 ml; soluble starch (1.5), soybean meal (1.5), glucose (0.5), NaCl (0.5), $CaCO_3$ (0.2), yeast extract (0.2), corn steep liquor (0.1 g; adjusted to pH 6.5] were inoculated with a culture of Y 90 3333 at a late stage of fungal growth. They were then incubated on a rotary shaker (200 rev min^{-1}; 48 h; 26 °C) to produce a seed culture. This was used (1% by volume) to inoculate flasks containing sterile production medium [200 ml; glucose (1.0), malt extract (2.0), peptone (1.0), Na_2HPO_4 (0.1 g $litre^{-1}$) and $ZnSO_4 \cdot 7H_2O$ (0.22), $CaCl_2$ (0.55), $MnCl_2 \cdot 4H_2O$ (0.5), $FeSO_4 \cdot 7H_2O$ (0.5), $CuSO_4 \cdot 5H_2O$ (0.16 mg $litre^{-1}$ in demineralised water); adjusted to pH 6.5 before autoclaving]. The flasks were then incubated on a rotary shaker (200 rev min^{-1}; 116 h). The fermentation was monitored by agar plate assays *in vitro* with *Neurospora crassa* Shear & Dodge,[6] *Botrytis cinerea* Pers ex Fr, *Fusarium culmorum* Sacc, *Pyricularia oryzae* Bri & Cavara and *Penicillium digitatum* (Pers) Sacc.

A 20-litre-scale fermentation, carried out in shake flasks, gave a mycelium cake which was filtered off from the whole-culture broth and extracted with acetone + methanol (1 + 1 by volume). Repeated chromatography of the concentrated extract on silica gel, with ethyl acetate + light petroleum as eluant, followed by preparative TLC on silica gel with ethyl acetate + light petroleum (3 + 7 by volume) as mobile phase, afforded ilicicolin E (8′9′-dehydroascochlorin, **1**).[7] The culture filtrate was loaded onto a bed of Diaion HP-20 and washed with water, followed by water containing increasing amounts of methanol.

Table 1. Activity *in planta* of the isolated compounds against *Phytophthora infestans*

	Activity[a]				
Dose (mg litre^{-1})	*Ilicicolin E (1)*	*Ilicicolin F (2)*	*Ilicicolin D (4)*	*Dechloroilicicolin D (3)*	*Ascofuranone (5)*
500	3	3	3	3	2
250	3	3	3	3	1
125	3	3	3	3	1

[a] From the separate experiments. On a scale 3 (good activity) to 1 (poor activity/inactive).

Pathogen	Host	Dose (mg litre^{-1})	Ilicicolin E	Ilicicolin F
Phytophthora infestans	Tomato	500	2	3
		50	2	3
Plasmopara viticola	Vines	500	3	3
		50	3	2
Erysiphe graminis f. sp. *triticae*	Wheat	500	1	1
		50	1	1
Magnaporthe grisea	Rice	500	2	2
		50	1	1
Pellicularia sasakii	Rice	500	2	1
		50	1	1
Botrytis cinerea	Tomato	500	1	1
		50	1	1
Stagonospora nodorum	Wheat	500	2	2
		50	1	1

Table 2. Extended screening results for ilicicolins E and F

Fractions obtained with methanol+water (8+2 by volume and with pure methanol were active against *Phytophthora infestans* (Mont) de Bary; that obtained with pure methanol, when subjected to repeated chromatography on silica gel, gave ilicicolin F (**2**), dechloroilicicolin D (cylindrol B)[8] (**3**) and a mixture of ilicicolin D (ascochlorin; **4**) and ascofuranone (**5**).[4] The components of this mixture were separated by TLC on silica gel using ethyl acetate+light petroleum (1+9 by volume) as mobile phase. Activity against *B cinerea* was also present in the fraction eluted from HP-20 with methanol+water (8+2 by volume) and this was shown to be due to the presence of the cyclic depsipeptide arthrichitin (**6**),[5] which was isolated from this fraction by MPLC on reverse-phase silica gel (RP-18) using step-gradient elution with water+methanol, followed by semi-preparative HPLC on RP-18 using an acetonitrile+water (60+40 by volume) isocratic system.

2.2 Biological testing

Tomato plants (*Lycopersicon esculentum* Mill cv First in the Field) were sprayed with the test material dissolved in methanol+water (1+1 by volume) containing Tween 20 (2.5 g litre^{-1}) as wetter, allowed to stand for 24 h and then inoculated with *P infestans* sporangia. The inoculated plants were kept in the dark for 24 h at 17–18 °C and 100% RH and then transferred to a growth chamber maintained at 17–18 °C and 90–95% RH with 12 h daylight per day. Disease severity was assessed five or six days after infection by comparison with infected, untreated control plants, using a scale 1 (poor activity/inactive) to 3 (good activity). Disease symptoms ranged from wrinkling of the leaves to necrotic lesions on the leaves and stem. More extended tests against seven pathogen/host combinations were carried out as above, except that the compounds were formulated in water+methanol (6+4 by volume).

3 RESULTS

The isolate 6 showed moderate activity *in planta* against *B cinerea* and *P viticola* at 500 mg litre^{-1} (data not shown).

The results of tests *in planta* with the compounds against *P infestans* are given in Table 1. The ilicicolins **1**–**4** showed good activity while ascofuranone was active only at 500 mg litre^{-1}. A mancozeb 800 g kg^{-1} WP used as a standard scored 3 at 100 mg AI litre^{-1}.

Ilicicolins E and F were isolated in sufficient quantities for extended testing, the results of which are shown in Table 2. Of the seven fungal species included in the extended screen the ilicicolins were mainly active against the two oomycetes *P infestans* and *P viticola*.

The present work confirms that the ilicicolins have interesting levels of fungicidal activity, particularly against the oomycetes and indicates that they may provide useful leads for further investigation.

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